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ACUTE INFECTION OF CHICKS AND CHRONIC INFECTION OF THE OVARIES OF HENS CAUSED BY THE FOWL-TYPHOID ORGANISM*

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INTRODUCTION

The earliest authentic descriptions of fowl typhoid are those of Klein[1] in 1889, in England, and of Moore[2] in 1895–96, in the United States. Klein designated the disease as infectious enteritis and the causative organism, *Bacillus gallinarum*. Moore called the disease infectious leukemia of fowls and the causative organism *Bacterium sanguinarium*.§ It has since been determined that these investigators studied the same disease, which is now known as fowl typhoid and has become recognized as an important cause of mortality of adult fowls throughout the world.

* A brief résumé of these studies was contained in the California Agr. Exp. Sta. Ann. Rpt. 1924–25: 72.

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§ In Bergey's Manual of Determinative Bacteriology (Williams and Wilkins Company, Baltimore, Maryland, 1923), *Bacterium sanguinarium* (Moore) is classified as *Eberthella sanguinaria* (Moore) and is said to be associated with cholera in chickens. The species of organisms stated to be associated with fowl typhoid are *Eberthella jeffersonii* (Hadley), *Eberthella pfaffi* (Hadley), and *Eberthella rettgeri* (Hadley). Klein's *Bacillus gallinarum* is classified as *Pasteurella avicida* (Perroncito), the cause of fowl cholera. This classification is apparently based largely upon the studies of Hadley reported in Rhode Island Agr. Exp. Sta. Bul. 174. This publication, however, presents *E. jeffersonii, E. pfaffi,* and *E. rettgeri* as new bacterial types, differing from both the fowl-cholera and fowl typhoid organisms. *Bact. sanguinarium* (Moore) and *B. gallinarum* (Klein) are regarded as identical and as the etiological agent of fowl typhoid. It would appear, therefore, that the fowl-typhoid organism is improperly classified in the above mentioned manual. For this reason *Bact. sanguinarium* (Moore), the name of the fowl-typhoid organism in common usage in the United States, is used in this paper.

Some investigators observed a marked similarity between *Bact. sanguinarium* and *Bact. pullorum,** the cause of bacillary white diarrhea of chicks. As a result, extensive comparative studies of the two species were made especially by Taylor,[3] Smith and Tenbreeck,[4] Rettger and Koser[5] Goldberg,[6] and Hadley.[7] These investigators concluded that the two organisms were indistinguishable in their serologic reactions but that there were sufficient differences in their action on carbohydrate media, in their other cultural characteristics, and in their morphology to establish the identity of the two species.

References in the literature to fowl typhoid are numerous. The disease, however, has nearly always been described as an acute infection of mature fowls, little consideration being given to the rôle that *Bact. sanguinarium* might play in causing mortality among young chicks.

The organism in recent years has been recovered from dead chicks by several investigators and it therefore can no longer be regarded of importance only in connection with fowl typhoid of adults. Panisset and Verge[8] in 1924 reported an epizootic among a small flock of chicks in France in which they isolated an organism closely resembling *Bact. sanguinarium.* Stafseth in Michigan, Bushnell in Kansas, and Beaudette in New Jersey have stated in personal communications that they have occasionally isolated *Bact. sanguinarium* from chicks which they suspected had died from bacillary white diarrhea. Beaudette[9] in 1925 reported the isolation of the organism both from young chicks and the ovary of a hen and he stated that 9 of 66 hens in the same flock reacted to an agglutination test with *Bact. pullorum* or *Bact. sanguinarium* antigens. In 1926, Doyle[10] reported similar observations regarding the occurrence of the infection in chicks and hens. He also stated that the examination of 140 eggs from 9 reacting hens showed them to be free from *Bact. sanguinarium.* Disease of baby chicks due to *Bact. sanguinarium* was first observed in this laboratory in May, 1921, in chicks submitted for diagnosis. Clinically and in all other respects the disease resembled bacillary white diarrhea. Since then the organism has been occasionally encountered in routine bacteriological examinations of chicks.

In November, 1924, opportunity was afforded to make a detailed study of an outbreak of disease due to *Bact. sanguinarium* in a lot of chicks which had been obtained for experimental purposes. The conditions under which the outbreak occurred made it seem possible that the infection was acquired by a transmission cycle identical with that of *Bact. pullorum.* Investigations were undertaken to determine whether this suspicion was well founded.

* Classified as *Salmonella pullora* in Bergey's Manual of Determinative Bacteriology, p. 218.

THE OUTBREAK OF DISEASE IN BABY CHICKS

On November 14, 1924, 145 chicks were obtained from a commercial hatchery for use in a coccidiosis control experiment. These chicks, all of which appeared vigorous on arrival, had been shipped in new boxes immediately upon removal from the incubator and were about thirty-six hours old when received. They were placed in pens which not only had been thoroughly disinfected but also had not previously contained poultry. The electric hovers, mash hoppers, and drinking fountains used were new and had also been disinfected. These chicks, therefore, were not exposed to infection of any kind except that which might have been present in the incubator or within or on the shell of the egg from which they were hatched.

The day following that on which the chicks were received, when they were about 60 hours old, the death of one chick occurred. *Bact. sanguinarium* was isolated in pure culture. Deaths from this cause continued and became so numerous that the coccidiosis control experiment for which the chicks were secured was abandoned. The outbreak of the disease due to *Bact. sánguinarium,* however, proved of equal interest and these chicks were, therefore, held for study and observation.

TABLE 1

RECORD OF MORTALITY AND RESULTS OF POST-MORTEM EXAMINATION OF ONE HUNDRED AND FORTY-FIVE CHICKS RECEIVED NOVEMBER 14, 1924

Time of death	Number died	Per cent died	Abnormal liver*		Unabsorbed yolk		*Bacterium sanguinarium* isolated		Bacteriological examination negative	
			Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent
First week	29	20.0	29	100.0	28	96.5	27	93.1	2	6.9
Second week	23	15.8	22	95.6	14	60.8	22	95.6	1	4.3
Third week	6	4.1	2	33.3	3	50.0	4	66.6	2	33.3
After third week	21	14.5	4	19.0	2	9.5	4	19.0	17	80.9
Total	79	54.4	57	72.1	47	59.5	57	72.1	22	27.8

* Abnormalities consisted of uniformly yellowish or mottled yellow and red discoloration or uniformly pale without any marked discoloration.

A careful autopsy and bacteriological examination was made of each chick that died. Microscopic, biochemic, and serologic methods were used for identification of cultures. Those that consisted of Gram-negative non-motile rods; that produced acid but no gas in dextrose,

mannite and maltose broth and did not ferment lactose and saccharose broth; and that were agglutinated by positive *Bact. pullorum* and *Bact. sanguinarium* serum but not by positive *B. avisepticus* serum were considered to be *Bact. sanguinarium*.

Table 1 gives a record of the mortality and results of post-mortem examination of the chicks which died before they were 45 days old.

DISCUSSION OF BABY CHICK MORTALITY

The mortality in this lot of 145 chicks during the first 45 days of their lives was 79, or 54.4 per cent. *Bact. sanguinarium* was isolated from 57, or 72.1 per cent, of those that died, or 39.3 per cent of the total number of chicks.

Forty-nine, or 85.9 per cent, of the deaths from fowl-typhoid infection occurred during the first two weeks. Failure to recover the organism was encountered in only 3 of the 52 chicks which died during this period. Of the 27 chicks which died after the second week, *Bact. sanguinarium* was recovered from 8, or 29.6 per cent.

The distribution of abnormal livers and unabsorbed yolks with respect to the age of the chicks at the time of death corresponded closely to the incidence of *Bact. sanguinarium* infection. Abnormalities of the liver were found in 51, or 98.0 per cent of the 52 which died during the first two weeks and in 6, or 22.2 per cent, of those which died later. Unabsorbed yolk was present in 42, or 80.7 per cent, of those which died during the first two weeks and in 5, or 18.5 per cent, of those which died later.

It can be seen that this outbreak of disease due to *Bact. sanguinarium* resembled in all respects bacillary white diarrhea of baby chicks due to *Bact. pullorum* infection.

OBSERVATIONS ON THE SURVIVORS

Twenty-five of the survivors, 20 females and 5 males, were kept for further study. An agglutination test with *Bact. sanguinarium* antigen was made on the blood serum of each bird when they were six, eight, and twelve months of age. No reactions occurred. One bird died when seven months old. No pathological changes were found in the ovary and a bacteriological examination was negative. One bird died when ten months of age. The port-mortem examination showed three small abnormal-appearing yolks in the ovary. The bacteriological examination of the liver, heart blood, and the three

yolks was negative. Since negative results were obtained from the three agglutination tests and no evidence of *Bact. sanguinarium* infection was found in the two birds which died, no further tests or examinations were made of the remaining birds.

While these studies failed to demonstrate that the survivors of an outbreak of fowl typhoid in baby chicks become chronic carriers of *Bact. sanguinarium* they do not preclude the possibility that some did become carriers, since only 25 of the 66 survivors were retained for observation.

INFECTION OF THE OVARIES OF HENS

As stated at the outset, the only possible sources of the infection in the chicks seemed to be either the incubators in which or the eggs from which they were hatched. Since the nursery trays and nursery tray cloths of the incubators were cleaned between hatches and little complaint of chick mortality had been experienced by the hatchery owner, the eggs seemed the most likely source of the infection. This suggested that chronic ovarian infection with *Bact. sanguinarium* might exist in breeding fowls and be transmitted to chicks through the egg in the same manner as *Bact. pullorum*. If this should prove to be true, it seemed possible that the carriers of the infection might be detected by means of the agglutination test. Permission was obtained to collect blood samples for the agglutination test from a portion of the flock of 1300 birds that produced the eggs from which the chicks were hatched.

On December 12, 1924, blood was drawn from 196 of the 1300 birds. The agglutination test was made on each blood sample with antigens prepared from both *Bact. pullorum* and *Bact. sanguinarium*. Positive reactions were obtained with 32 or 16.3 per cent.

The degree of agglutinations obtained with the positive sera is given in table 2.

An analysis of table 2 shows:

1. Partial or complete agglutination with both antigens was obtained with 29 of the 32 samples.

2. Complete agglutination with both antigens in at least one dilution was obtained with 22 samples.

3. In three instances (Nos. 444, 461, 479) there was complete agglutination with *Bact. pullorum* antigen but only partial with *Bact. sanguinarium* antigen. Repetition of the tests with these samples gave the same results.

4. One sample (No. 416) gave complete agglutination with *Bact. sanguinarium* antigen but only partial agglutination with *Bact. pullorum* antigen. Repetition of the test with this sample gave the same result.

TABLE 2

THE REACTIONS TO THE AGGLUTINATION TEST OF THE POSITIVE SERA

Bird No.	*Bact. pullorum* antigen		*Bact. sanguinarium* antigen		Bird No.	*Bact. pullorum* antigen		*Bact. sanguinarium* antigen	
	0.02 mil serum	0.01 mil serum	0.02 mil serum	0.01 mil serum		0.02 mil serum	0.01 mil serum	0.02 mil serum	0.01 mil serum
304	+	∓	+	−	395	+	+	+	+
315	+	±	+	±	415	+	−	+	−
320	±	+	±	±	416	∓	−	+	−
325	±	−	∓	−	423	+	∓	−	−
341	±	±	±	±	425	±	+	±	±
349	+	−	−	−	432	∓	∓	±	−
350	+	−	−	−	435	+	∓	+	∓
354	±	±	±	±	443	+	+	+	±
365	+	+	±	±	444	±	+	±	−
368	±	+	±	+	452	±	±	±	±
371	±	±	±	±	456	±	±	±	±
373	+	−	±	±	461	+	±	∓	−
381	±	+	±	±	464	+	+	±	±
382	±	±	±	+	465	+	∓	+	∓
383	+	−	+	+	477	∓	∓	∓	−
394	±	+	+	+	479	+	+	∓	∓

Explanation of symbols in table:

Two readings were made of each test at the expiration of 24 and 48 hours, respectively. Only final readings were entered.

Two dilutions were made by adding 0.02 mil and 0.01 mil of serum respectively, to 1 mil of antigen.

− indicates no evidence of reaction.

∓ indicates slight sediment but supernatant fluid turbid.

± indicates more sediment than ∓ but still a faint cloudiness in supernatant fluid.

± indicates that after over-night incubation complete agglutination is present.

+ indicates that after over-night incubation there was not complete agglutination, but on standing for 24 hours longer the fluid cleared up.

5. Partial agglutination with both *Bact. pullorum* and *Bact. sanguinarium* antigens was obtained with three samples (Nos. 325, 432, 477). Repetition of the tests with these samples gave the same results.

6. Complete agglutination with *Bact. pullorum* antigen but *none* with *Bact sanguinarium* antigen was obtained with three samples (Nos. 349, 350, 423). Repetition of the tests with these samples gave the same results.

The results of the agglutination test indicated that the reacting birds were infected either with both *Bact. pullorum* and *Bact. sanguinarium* or with one of the two species alone, but did not make it possible to arrive at a more definite conclusion. In order that information on this point might be obtained 29 of the 32 reactors were secured

for autopsy. The three reactors not obtained were numbers 315, 435, and 452, all of which had reacted positively with both antigens. The birds obtained were examined for abnormalities, particularly of the ovary. Cultures were made from the livers, spleens, ovaries, and normal and abnormal-appearing ovules and yolks. The results of the autopsies and bacteriologic examinations appear in table 3.

DISCUSSION OF POST-MORTEM AND BACTERIOLOGICAL FINDINGS

Abnormal ovaries were found in all of the twenty-nine reacting hens examined. The abnormalities of the ovaries included bloody or caseated ovules; yolks varying from a pea to a hazelnut in size with a thick opaque capsule containing yellow semi-solid oily material or a clear yellow oily liquid with white flakes in suspension; small partially solidified, blood-tinged yolks; yolks of various sizes with capsule but partially filled with a thick yellow or greenish-yellow liquid; solidified angular yellow or greenish-yellow or blood-tinged yolks; and a number of small cysts attached to the ovary.

No correlation was found to exist between the degree of reaction to agglutination test of the blood serum of the birds and the extent of ovarian abnormalities present. For example, serum from bird 395 in which only slight ovarian abnormalities were found, caused complete agglutination in both dilutions with both antigens, while serum from bird 416, in which extensive abnormalities of the ovaries were found, produced partial agglutination in the 1–50 dilution with the *pullorum* antigen, complete agglutination in the 1–50 dilution with the *sanguinarium* antigen, but no agglutination in the 1–100 dilution with either antigen.

In addition to abnormalities of the ovaries, in seven birds yolk material was found in the abdominal cavity. In three of these (365, 416, 456) the material had the appearance of having escaped from a recently ruptured yolk. In the other four cases (341, 350, 383, 423) the material was solidified or encapsulated indicating that it had been in the abdominal cavity for some time. In two other birds (354, 464) there was no free yolk material in the abdominal cavity but the peritoneum was thickened and opaque suggesting that rupture of a yolk had previously occurred in these birds. The owner stated that a number of the flock had died from ruptured yolk during the preceding laying season. None of the latter were given a bacteriological examination so it is not known that *Bact. sanguinarium* was associated with

TABLE 3

RESULTS OF EXAMINATIONS OF REACTING HENS

Bird No.	Agglutination test reaction		Condition of ovary	Description of abnormal yolks	Culture made from	Growth obtained from	Organism recovered
	Bact. pullorum	*Bact. sanguinarium*					
304	+∓	+−	Dormant	1 very bloody ovule	Liver, spleen, 1 normal ovule, 1 bloody ovule, ovary	None	None
320	±+	±±	Dormant	1 small yolk, capsule thick and opaque, contents yellow and semi-solid	Liver, spleen, 1 abnormal yolk	Abnormal yolk	*Bact. sanguinarium*
325	±−	∓−	Dormant	2 small semi-solid, blood-tinged yolks	Liver, spleen, 2 abnormal yolks	2 abnormal yolks	*Bact. sanguinarium*
341	±±	±±	Dormant	3 encapsulated masses of yolk in abdominal cavity. 2 yolks with thick opaque capsules containing clear yellow oily liquid. 1 with capsule not filled, contents greyish-yellow in color. Several other small yolks	Liver, spleen, 3 encysted abdominal yolks, 2 abnormal yolks	Abdominal yolks, abnormal yolks	*Bact. sanguinarium*
349	+−	−−	Dormant	Several small yolks yellowish green in color, contents liquid, capsule not filled	Liver, spleen, 4 abnormal yolks	2 abnormal yolks	*Bact. sanguinarium*
350	+−	−−	Active	1 encapsulated mass of yolk size of a hazelnut in abdominal cavity, contents greyish-yellow thick liquid, numerous minute caseated ovules. Several pea-sized abnormal yolks, capsules not filled, contents yellow liquid	Liver, spleen, 4 caseated ovules, egg in abdominal cavity, 2 abnormal yolks, 2 normal yolks	2 abnormal yolks	*Bact. sanguinarium*
354	±±	±±	Dormant	1 solidified, angular, and blood-tinged yolk. 1 large, bloody yolk, capsule not filled, contents liquid. 2 small yolks, contents clear, yellow oily liquid, containing white flakes, capsule opaque	Liver, spleen, 4 abnormal yolks	2 abnormal yolks	*Bact. sanguinarium*

TABLE 3—(*Continued*)

Bird No.	Agglutination test reaction		Condition of ovary	Description of abnormal yolks	Culture made from	Growth obtained from	Organism recovered
	Bact. pullorum	*Bact. sanguinarium*					
365	++	±±	Active	Free liquid yolk material in abdominal cavity. 2 abnormal yolks, capsules not filled, yellow liquid contents. 2 small angular solidified yolks	Liver, spleen, abdominal yolk, 4 abnormal yolks	Abdominal yolk, solidified yolks	*Bact. sanguinarium*
368	±+	±+	Dormant	1 entire egg in abdominal cavity, contents creamy, foul odor. Numerous normal appearing ovules	Liver, spleen, abdominal egg, ovules, ovarian tissue	Egg, ovarian tissue, ovules	*Staphlococcus* from egg. *Bact. sanguinarium* from ovary and ovule
371	±±	±±	Active	1 solidified yolk. Several small yolks, capsules not filled, contents liquid	Liver, spleen, 3 abnormal yolks, 1 normal yolk	1 abnormal yolk	*Bact. sanguinarium*
373	+−	±±	Dormant	2 yolks, hazelnut size; capsules thick and opaque, contents clear, yellow, oily liquid with white flakes, 2 yolks, capsules not filled; contents yellow liquid. 1 yolk, capsule not filled, contents bloody	Liver, spleen, 5 abnormal yolks	All 5 yolks	*Bact. sanguinarium*
381	±+	±±	Dormant	Several yolks size of large pea, contents solidified and blood-tinged; 1 slightly larger, contents greyish yellow color and viscid	Liver, spleen, 3 solidified yolks, 1 liquid yolk	3 solidified yolks	*Bact. sanguinarium*
382	±±	±+	Dormant	One half-size yolk, greenish brown, liquid contents, capsule not filled. Several caseated ovules, 3 to 4 mm. in diameter. 2 small semi-solid yolks, capsules not filled, greyish brown in color	Liver, spleen, large yolk, 2 small yolks, ovules	2 small yolks	*Bact. sanguinarium*
383	+−	++	Dormant	1 large and several small solidified angular yolks. Caseated material in abdominal cavity, probably from ruptured yolk. Peritonitis	Liver, spleen, yolk in abdominal cavity, large and small abnormal yolks	Large and small abnormal yolks	*Bact. sanguinarium*

TABLE 3—(*Continued*)

Bird No.	Agglutination test reaction		Condition of ovary	Description of abnormal yolks	Culture made from	Growth obtained from	Organism recovered
	Bact. pullorum	*Bact. sanguinarium*					
394	±+	++	Active	Numerous small cysts in ovary	Liver, spleen, ovary, ovarian cysts	Cysts	*Bact. sanguinarium*
395	++	++	Active	2 small yolks, capsules not filled	Liver, spleen, 2 abnormal yolks, 1 normal yolk	None	None
415	+−	+−	Dormant	2 small yolks, capsules thick and opaque, contents semi-solid and bloody	Liver, spleen, 2 abnormal yolks	Abnormal yolks	*Bact. sanguinarium*
416	∓−	+−	Active	Blood-tinged liquid yolk in abdominal cavity. Several pea-sized abnormal yolks, contents clear yellow oily liquid with white flakes. 1 large abnormal yolk, capsule not filled, contents yellow liquid	Liver, spleen, abdominal yolk, 2 small and 1 large abnormal yolks, 1 normal yolk	1 abnormal yolk	*Bact. sanguinarium*
423	+∓	−−	Dormant	Small encysted mass of solidified bloody yolk in abdomen. One abnormal yolk, capsule not filled, contents yellow thick liquid	Liver, spleen, abdominal yolk, abnormal yolk	Abnormal yolk	*Bact. sanguinarium*
425	±+	±±	Active	Three half-size abnormal yolks, capsules thickened, contents partially solidified. 1 very bloody, others yellow in color	Liver, spleen, normal yolk, 3 abnormal yolks	2 abnormal yolks	*Bact. sanguinarium*
432	∓∓	±−	Dormant	1 hazelnut-sized abnormal yolk, capsule not filled, contents yellow liquid. Several small abnormal yolks of same character	Liver, spleen, 2 abnormal yolks	Both abnormal yolks	*Bact. sanguinarium*
443	++	+±	Active	1 yolk 1 cm. diameter, capsule thick opaque, contents yellow, solidified, 1 yolk 5 mm. in diameter, capsule not filled, contents semi-solid and yellow	Liver, spleen, abnormal yolks, normal yolks	Abnormal yolks	*Bact. sanguinarium*

TABLE 3—(*Concluded*)

Bird No.	Agglutination test reaction		Condition of ovary	Description of abnormal yolks	Culture made from	Growth obtained from	Organism recovered
	Bact. pullorum	*Bact. sanguinarium*					
444	±+	±−	Active	1 large yolk, capsule not filled, contents thick yellow liquid. 2 large yolks, capsules not filled, contents thick blood-tinged liquid. 1 hazelnut size yolk, capsule thick, opaque, contents clear yellow, oily liquid with white flakes	Liver, spleen, 4 abnormal yolks, 1 normal yolk	4 abnormal yolks	*Bact. sanguinarium*
456	±±	±±	Active	Liquid yolk in abdominal cavity. 1 yolk, capsule not filled, contents thick yellowish brown liquid. 1 yolk solidified, angular, yellowish brown. 3 yolks, capsules thick, opaque, contents clear yellow oily liquid with white flakes	Liver, spleen, abdominal yolk, abnormal yolks	All abnormal yolks	*Bact. sanguinarium*
461	+±	∓−	Active	1 large, solidified angular bloody yolk. 2 yolks hazelnut-size, capsules thick, opaque, contents solidified, yellow in color. 1 hazelnut-size, capsule thick, opaque, contents yellow, oily liquid with white flakes. Numerous small abnormal ovules	Liver, spleen, 4 large abnormal yolks	Four abnormal yolks	*Bact. sanguinarium*
464	++	±±	Active	1 small yolk, capsule not filled, contents thick yellow liquid	Liver, spleen, abnormal yolk, normal yolk, ovary	Abnormal yolk	*Bact. sanguinarium*
465	+∓	+∓	Dormant	Hazelnut-size, greenish-brown yolk; capsule not filled. Several small caseated ovules	Liver, spleen, abnormal yolk, ovules	Abnormal yolk	*Bact. sanguinarium*
477	∓∓	∓−	Dormant	Hazelnut-sized irregular-shaped yolk with opaque capsule, containing yellow pasty material	Liver, spleen, abnormal yolk	Abnormal yolk	*Bact. sanguinarium*
479	++	∓∓	Active	1 large yolk, capsule not filled, contents greyish-yellow thick liquid; 1 large angular yolk, contents partially solidified	Liver, spleen, normal yolks, abnormal yolks	Abnormal yolks	*Bact. sanguinarium*

the losses at that time. However, since *Bact. sanguinarium* is frequently found associated with ruptured yolk, and ovarian infection with the organism was found to exist in the flock, it seems possible that *Bact. sanguinarium* was present at the time the deaths from ruptured yolk occurred and that the ovarian infection may have become established at that time.

Bact. sanguinarium was isolated from abnormal yolks, ovules, or cysts in all except 2 of the 29 birds. Included with those from which *Bact. sanguinarium* was isolated were the three birds (Nos. 349, 350, 423) whose blood serum had given a positive agglutination reaction with *Bact. pullorum* antigen and a negative reaction with *Bact. sanguinarium* antigen. The abnormalities of the ovaries in the two birds (Nos. 304 and 395) from which *Bact. sanguinarium* was not isolated, were very slight, consisting of one small, bloody ovule in bird No. 304 and two small, flabby yolks in bird No. 395. Failure to isolate *Bact. sanguinarium* from these two cases does not necessarily prove, however, that the organism was not present, since it is possible that it was present and that we failed to recover it in cultures.

GENERAL DISCUSSION

These studies demonstrate that *Bact. sanguinarium* may produce an acute, highly fatal disease of young chicks and a chronic infection of the ovaries of hens which cannot be differentiated from disease of chicks and ovarian infection of hens caused by *Bact. pullorum,* except by the difference in the cultural characteristics of the organism isolated from affected birds. Agglutinins occur in the blood serum of hens that are infected with *Bact. sanguinarium.* However, the ordinary routine agglutination test does not serve to differentiate between ovarian infection with *Bact. sanguinarium* and *Bact. pullorum* because serum from a hen infected with the former will cause agglutination of antigens prepared from either of the two species of organisms. This cross-agglutination makes it possible to detect carriers of either *Bact. sanguinarium* or *Bact. pullorum* by an agglutination test employing *Bact. pullorum* antigen. It may, therefore, be considered as enhancing rather than detracting from the value of the agglutination test in the detection of adult hens that harbor infection that may be transmitted through the medium of eggs to offspring.

Although *Bact. sanguinarium* was not isolated from eggs laid by infected hens, these studies furnish evidence that this organism, like *Bact. pullorum,* is transmitted directly to chicks through eggs laid

by infected adults. Deaths among the chicks from *Bact. sanguinarium* infection began when they were 60 hours old. It seems quite certain that the chicks did not acquire the infection from contaminated brooder houses or hovers and very unlikely that the infection originated in the incubators or shipping boxes. The only remaining source of infection is the parent stock among which chronic ovarian infection with *Bact. sanguinarium* was found to exist. It seems probable, therefore, that some of the eggs laid by these hens contained *Bact. sanguinarium,* which resulted in infection of the chicks hatched from them.

The origin of chronic infection of the ovaries of the hens with *Bact. sanguinarium* remains undetermined. It has been found that chicks that survive an outbreak of disease due to infection with *Bact. pullorum* may continue to harbor the infection and that it usually becomes localized in the ovaries. Observations made on 25 of the 66 chicks that survived the infection with *Bact. sanguinarium* and were kept for one year, however, failed to show that any of them had become carriers of the organism. It is in this particular only that these studies fail to show that the behavior of *Bact. sanguinarium* in either chicks or adult fowls may be the same as that of *Bact. pullorum.* The facts that losses from ruptured yolk had occurred in the flock of hens a year before the studies herein reported were made and that *Bact. sanguinarium* infection is frequently associated with ruptured yolk, suggest that the ovarian infection with *Bact sanguinarium* may have then become established. However, no bacteriological examination of dead birds from this flock had ever been made and it was not known that *Bact. sanguinarium* existed in it before the agglutination tests were made.

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